

CLAIMS

1. A modified polypeptide having carbohydrate processing enzymatic activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 comprising a mutation in at least one of W433, E432 and M439;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase, comprising at least one mutation at an amino acid residue equivalent to W433, E432 or M439 of SEQ ID NO:2; and
- (c) a variant of (a) or (b) having carbohydrate processing enzymatic activity and comprising at least one amino acid mutation at a position equivalent to W433, E432 or M439 of SEQ ID NO:2.

2. The polypeptide according to claim 1 in which the mutation is selected to broaden the substrate specificity of the polypeptide compared to a polypeptide not so modified.

3. The polypeptide according to claim 1, wherein the mutation is an amino acid substitution.

4. The polypeptide according to claim 1 in which the polypeptide comprises:

- (i) SEQ ID NO:2 having one or more of W433, E 432 and M439 substituted by cysteine, valine or alanine; or
- (ii) the amino acid sequence as defined in (b) or (c) having one or more of the amino acid residues equivalent to W433, E432 or M439 substituted by cysteine, valine or alanine.

5. A modified polypeptide having carbohydrate processing enzymatic activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 comprising one or more mutations selected from the group consisting of W433C, E432C and M439C;
  - (b) the amino acid sequence of a family 1 glycosyl hydrolase, comprising at least one mutation at an amino acid equivalent to W433, E432 or M439 in SEQ ID NO:2 wherein the amino acid is substituted by a C (cysteine) residue; and
  - (c) a variant of (a) or (b) having carbohydrate processing enzymatic activity and comprising at least one mutation at an amino acid equivalent to W433, E432 or M439 in SEQ ID NO:2 wherein the amino acid is substituted by a C (cysteine) residue.
6. The polypeptide according to claim 5, wherein the C residue introduced by the mutation is chemically modified.
7. The polypeptide according to claim 6, wherein the C residue is modified so as to comprise a positively-charged group.
8. The polypeptide according to claim 7, wherein the positively charged group is of formula  $-(CH_2)_n-N^+R_3$ , wherein n is a positive integer from 1 to 4 and each R, which may be the same or different, is H or a C<sub>1</sub>-C<sub>4</sub> alkyl group.
9. The polypeptide according to claim 8, wherein the positively charged group is  $-CH_2CH_2NMe_3^+$ .
10. The polypeptide according to claim 6, wherein the C residue is modified so as to comprise a negatively-charged group.
11. The polypeptide according to claim 10, wherein the negatively-charged group is of formula  $-(CH_2)_n-SO_3^-$  or  $-(CH_2)_n-COO^-$ , wherein n is a positive integer from 1 to 4.

12. The polypeptide according to claim 11, wherein the negatively-charged group is of formula  $-\text{CH}_2\text{CH}_2-\text{SO}_3^-$ .
13. The polypeptide according to claim 6, wherein the C residue is modified so as to comprise an uncharged group.
14. The polypeptide according to claim 13, wherein the uncharged group is a  $\text{C}_1\text{-C}_4$  alkyl group.
15. The polypeptide according to claim 14, wherein the uncharged group is methyl.
16. The polypeptide according to claim 1, which further comprises a mutation of a catalytic nucleophilic residue of the active site.
17. The polypeptide according to claim 16, wherein the further mutation is:
  - (i) E387A or E387G in SEQ ID NO:2 or
  - (ii) substitution of E387 with A or G in the amino acid sequence as defined in (b) or (c) of claim 1.
18. The polypeptide according to claim 1, wherein the polypeptide has glycosyl synthase, glycosyl hydrolase, and/or transglycosylase activity.
19. The polypeptide according to claim 1, wherein the family 1 glycosyl hydrolase is *Sulfolobus solfataricus*  $\beta$ -glycosidase.
20. The polypeptide according to claim 6, which further comprises a mutation of a catalytic nucleophilic residue of the active site.
21. The polypeptide according to claim 20, wherein the further mutation is:

- (i) 387A or E387G in SEQ ID NO:2 or
- (ii) substitution of E387 with A or G in the amino acid sequence as defined in (b) or (c) of claim 5.

22. The polypeptide according to claim 6, wherein the polypeptide has glycosyl synthase, glycosyl hydrolase, and/or transglycosylase activity.

23. The polypeptide according to claim 6, wherein the family 1 glycosyl hydrolase is *Sulfolobus solfataricus*  $\beta$ -glycosidase.

24. A polynucleotide encoding the polypeptide having carbohydrate processing enzymatic activity according to claim 1.

25. An expression vector comprising the polynucleotide according to claim 24.

26. A host cell transformed with the vector according to claim 25.

27. A method for hydrolysing a  $\beta$ -glycoside, synthesising a  $\beta$ -glycoside or transglycosylation, which method comprises contacting a glycoside substrate with a modified polypeptide having carbohydrate processing enzymatic activity, said polypeptide comprising an amino acid sequence selected from:

(a) the amino acid sequence of SEQ ID NO:2 comprising a mutation in at least one of W433, E432 or M439;

(b) the amino acid sequence of a family 1 glycosyl hydrolase, comprising at least one mutation at an amino acid residue equivalent to W433, E432 or M439 of SEQ ID NO:2; and

(c) a variant of (a) or (b) having carbohydrate processing enzymatic activity and comprising at least one amino acid mutation at a position equivalent to W433, E432 or M439 of SEQ ID NO:2.

28. The method according to claim 27, wherein the glycoside substrate is selected from the group consisting of a glucoside, a galactoside, a fucoside, a xyloside, a mannoside, and a glucuronide.

29. The method according to claim 27, wherein the polypeptide is contacted with a sample containing at least two different glycosides.

30. A method for hydrolysing a  $\beta$ -glycoside, synthesising a  $\beta$ -glycoside or transglycosylation, which method comprises contacting a glycoside substrate with a modified polypeptide having carbohydrate processing enzymatic activity, said polypeptide comprising an amino acid sequence selected from:

(a) the amino acid sequence of SEQ ID NO:2 comprising one or more mutations selected from the group consisting of W433C, E432C and M439C;

(b) the amino acid sequence of a family 1 glycosyl hydrolase, comprising at least one mutation at an amino acid equivalent to W433, E432 or M439 in SEQ ID NO:2 wherein the amino acid is substituted by a C (cysteine) residue; and

(c) a variant of (a) or (b) having carbohydrate processing enzymatic activity and comprising at least one mutation at an amino acid equivalent to W433, E432 or M439 in SEQ ID NO:2 wherein the amino acid is substituted by a C (cysteine) residue; wherein the C residue introduced by the mutation of (a), (b) or (c) is chemically modified.

31. The method according to claim 30, wherein the glycoside substrate is selected from the group consisting of a glucoside, a galactoside, a fucoside, a xyloside, a mannoside, and a glucuronide.

32. The method according to claim 30, wherein the polypeptide is contacted with a sample containing at least two different glycosides.